

## The frequency of human papillomaviruses in colorectal cancer samples in Mashhad, northeastern Iran

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**Background/aim:** Infection with the human papillomaviruses (HPVs) is associated with the development of several cancers, including oral, esophageal, skin, lung, and cervical. However, the association of HPVs and colorectal cancers remains controversial. The aim of this study was to evaluate the association between HPV infection and paraffin-embedded colorectal tissue samples in Mashhad in the northeast of Iran.

**Materials and methods:** Paraffin-embedded tissue specimens from 111 patients with colorectal cancer were subjected to DNA extraction. The quality of extracted DNA was confirmed by amplification of a  $\beta$ -globin fragment using polymerase chain reaction (PCR) and GH20/PCO4 primers. PCR with GP5+/GP6+ primers was then performed on positive samples to evaluate the sequence of HPVs.

**Results:** A total of 100 colorectal samples with positive results for the  $\beta$ -globin gene were analyzed. The age of patients ranged from 18 to 72 years (mean: 52). Sixty-four patients (56.7%) were male and 47 patients (43.4%) were female. One (1%) out of 100 patients with colorectal cancer was found to be positive for HPV DNA.

**Conclusion:** Results of the current study suggested that HPV infection is not common in patients with colorectal cancer in our population. We concluded that HPV types that are associated with malignant transformations do not meaningfully contribute to adenocarcinoma of the colon among our population.

**Key words:** Human papillomaviruses, colorectal cancer, paraffin-embedded specimens

### 1. Introduction

In 2008, colorectal cancer was reported to be the second and third most common type of malignant cancers among women and men, respectively. In 2008, globally, of about 1.23 million new cases of colon cancers, about 608,000 people died (1). According to some heredity studies, about 20% of patients who suffer from this type of cancer have a family history. The presence of at least 2 patients suffering from colorectal cancer in a family accelerated the risk of incidence by 2 to 3 times (2), whereas some other studies showed that 75% to 95% of the cases of colon cancers were found in people who had no genetic history of this type of cancer. Some other factors such as age and sex are also suggested to have a role. From this point of view, older men are more susceptible to develop this type of cancer (3).

Some genetic factors that are assumed to be involved are as follows (4): reduction in the activity of the APC tumor suppressor gene functionally or physically; the *K-RAS* gene, which encodes some molecules that could further bind to active or inactive guanosine triphosphate; reduction in the activity of the tumor suppressor gene at 18q21; and late stage mutagenesis of the TP53 gene in colorectal cancers.

Currently, the roles of some viruses, particularly papillomaviruses, on colorectal cancers are under investigation. These viruses can cause malignancy both in animals and humans. Papillomaviruses are small viruses lacking an envelope. They have nucleocapsids with icosahedral symmetry and are 52–55 nm in size. Its capsid contains 72 capsomeres. Density of virus particles in CsCl is about 1.34 g/mL. The human papillomavirus (HPV)

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encodes 8 proteins. Six of these proteins are from the first region of their genome (E1–E7) and are nonstructural, while the rest (L1 and L2) encode the proteins of the main capsid. L1 is a highly conserved protein in all types of papillomaviruses and L2 has a key role in the aggregation of the viral capsid. Humans are the only host for papillomaviruses. HPVs attack the skin, oral mucosa, pharynx, trachea, and mucosa of the bronchi. According to some recent studies, HPVs may also be involved in esophageal carcinogenesis. Between 1995 and 2011, many findings showed the association of HPV and colon cancer (5–12); however, some other studies revealed that there was no significant relationship between HPV and colorectal cancers (13–15). The aim of this study was to evaluate the frequency of HPVs in patients with colorectal cancer in Mashhad, in the northeast of Iran.

## 2. Materials and methods

### 2.1. Sample collection and DNA extraction

Colorectal carcinoma specimens were obtained from patients who were referred to the Pathology Department of Imam Reza Hospital and Qaem Hospital in Mashhad, Iran. After approval of the study by the research ethics committee and informed written consent by patients, a total of 111 colorectal carcinoma specimens were obtained. Five sections of 10–20  $\mu\text{m}$  of the paraffin-embedded samples were prepared and the xylene-ethanol method was used for deparaffinization of the samples. Tissue digestion was performed using digestion buffer, which consisted of Tris-Cl (100 mM, pH 7.5) and Tween-20 (0.05%) as described previously (16). Prepared samples were used directly for polymerase chain reaction (PCR) amplification without more purification steps.

### 2.2. PCR of $\beta$ -globin gene used to check the quality of extracted DNA

In the current study, the quality of extracted DNA from paraffin-embedded tissue samples was determined by the amplification of a 260-bp fragment of the  $\beta$ -globin gene as an internal control. The PCO4 and GH20 primers were used for the PCR amplification. The PCR reaction mixture contained 0.8  $\mu\text{L}$  (~1  $\mu\text{g}$ ) of DNA, 0.4  $\mu\text{L}$  of dNTP (10 mM), 0.8  $\mu\text{L}$  of Taq DNA polymerase (5 U/ $\mu\text{L}$ , CinnaGen, Iran), 2.5  $\mu\text{L}$  of 10X PCR buffer, 1.6  $\mu\text{L}$  of  $\text{MgCl}_2$  (25 mM), and 10 pmol/ $\mu\text{L}$  of each primer, in a total reaction volume of 25  $\mu\text{L}$ . The PCR amplification was performed for 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s) after an initial denaturation step of 94 °C for 5 min. The cycles were followed by a 5-min extension at 72 °C. The PCR product was visualized on a 1.5% agarose gel by green viewer (Pars Tous, Iran) staining and UV photography.

In order to determine the type of HPV in the extracted DNA samples, GP5+ (5'-TTTGTACTGTGGTAGATACTAC-3') and GP6+

(5'-GAAAATAAACTGTAAATCATATTC-3') primers were used to amplify a 142-bp fragment of L1 (a highly conserved sequence of HPV gene). By performing this PCR, almost all HPV types, including high- and low-risk types, could be identified. The PCR reaction mixture consisted of 3  $\mu\text{L}$  of DNA (100 ng/ $\mu\text{L}$ ), 0.4  $\mu\text{L}$  of dNTP (10 mmol), 0.3  $\mu\text{L}$  of Taq DNA polymerase (5 U/ $\mu\text{L}$ , CinnaGen, Iran), 0.6  $\mu\text{L}$  of  $\text{MgCl}_2$  (50 mmol), and 10 pmol/ $\mu\text{L}$  of each primer, in a total volume of 25  $\mu\text{L}$ . The PCR program was as follows: denaturation for 5 min at 94 °C; 35 cycles of 94 °C for 45 s, 47.5 °C for 45 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min. Fragment sizes of PCR products were determined by comparison with appropriate molecular weight marker in a 1.5% agarose gel.

### 2.3. Statistical analysis

Data were analyzed with SPSS 18 (SPSS Inc., Chicago, IL, USA) and Fisher's exact test. A P-value below 0.05 was considered statistically significant.

## 3. Results

One hundred samples showed a 260-bp fragment in the PCR. The positive samples were subjected to amplification of a fragment of the highly conserved region of the L1 gene of HPV (HPV L1). The results indicated that among 100 samples, only 1 sample (1.0%) showed a 142-bp fragment of HPV L1. This sample could be considered as an HPV-positive sample. Patients' ages ranged between 18 and 72 years and the mean age was 52. Among the patients, 64 (56.7%) were male and 47 (43.4%) were female. The observed patient infected with HPV was a 60-year-old woman. There was a relationship between the frequency of patients with colorectal carcinoma and increase in age.

## 4. Discussion

Today colorectal cancer is considered as a common cancer and many studies have been performed in this regard. In 2011, Lorenzo et al. performed a metaanalysis study of some papers that had been published during the last 2 decades about the relationship between HPV and colorectal cancer. They found that there was an association between HPV and tumors (11). Liu et al. investigated the prevalence of HPV in a Chinese population suffering from colorectal cancer. Their results showed that the frequency of HPV DNA found in tumor tissues was higher than in nontumor colorectal tissues and peripheral blood samples ( $P < 0.001$ ) (10).

In a study performed by Damin et al. in 2007, the role of HPV infection in colorectal cancer and prognostic factors were evaluated. Their results showed that HPV DNA was positive in 60 (83.3%) of the total 72 cancerous colorectal samples. However, no HPV DNA was observed in any of the noncancerous tissues ( $P < 0.001$ ) (9).

In another study, cancerous and normal tissues were studied to assess the relationship between HPV DNA and colorectal cancers. HPV DNA was found in 84% of cancerous and 53% of normal mucosal tissues (7). In 1995, Cheng et al. investigated the relationship between HPV and colorectal cancer and their findings confirmed this relationship (8). In 2010, Gornick et al.'s studies on frizzed tumor tissues showed that HPV genotypes do not have a role in adenocarcinoma colon cancer (13). This finding, which is in agreement with the current study, suggested that the prevalence of HPV was low in patients with colorectal cancer. They also showed that different types of HPV that are usually associated with malignant changes were not found in patients who had suffered from colon adenocarcinoma.

Although several studies confirmed the relationship between HPV and colorectal cancer, others did not support the association. This may have been caused by genetic, geographical, and cultural differences of the patients in different studies.

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